Alkaline Phosphatase Assay Kit

Catalog No.: abx096002

Size: 96 tests

Detection Range: 0.13 King unit/100 ml – 50 King unit/100 ml

Sensitivity: 0.13 King unit/100 ml

Storage: Store all liquid components at 4°C in the dark.

Application: For quantitative detection of alkaline phosphatase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

Introduction

Alkaline phosphatases (ALPs) are a group of enzymes that catalyze the hydrolysis of phosphate esters at alkaline pH. In mammals, ALP is primarily found in the liver, kidney, and bone. High serum ALP concentrations are associated with primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), sarcoidosis, malignant biliary obstruction, and hepatic lymphoma.

Abbexa's Alkaline Phosphatase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Alkaline phosphatase activity. Alkaline phosphatase decomposes benzene disodium phosphate to produce phenol and phosphoric acid. Phenol reacts with 4-aminopyrline in an alkaline solution, it is then oxidized with potassium ferricyanide to form a red quinone biological pigment. The concentration of the reaction product can then be calculated indirectly by measuring the absorbance at 520 nm.

Kit components

1. 96-well microplate
2. Buffer Solution: 3 ml
3. Substrate: 3 ml
4. Chromogenic Reagent: 18 ml
5. Standard (0.5 mg/ml): 1.5 ml
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (520 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly. If not tested within 24 hours, serum/ plasma samples can be stored for up to 1 month at -80°C.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue, and wash in cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl of normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4) and homogenize manually, using a dounce homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes to remove insoluble material. Collect the supernatant, keep on ice and assay immediately.
- **Cell lysates/ Cell culture supernatants:** Harvest at least 1 × 10^6 cells and wash with PBS (0.01 M, pH 7.4). Per 1 × 10^6 cells, add 300 – 500 µl of normal saline (0.9 % NaCl) OR PBS (0.01 M, pH 7.4) and Homogenize manually with an ultrasonic cell disruptor at 4°C.Centrifuge at 10,000 × g for 10 minutes. Collect supernatant, keep on ice, and assay immediately.

Note: To calculate Alkaline phosphatase activity in tissue homogenates and cell samples using the formulae in section **C. Calculation of Results.** The protein content of the supernatant should be determined separately **(abx097193)**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human plasma	1
Human urine	1
Rat serum	1
Cell culture supernatant	1
10% Mouse kidney tissue homogenate	30 – 50
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
Hepg2 cells	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

Allow all reagents to equilibrate to room temperature before use.

- **Working Solution:** Prepare enough Working Solution for each well used in the assay. Per well, mix thoroughly 25 µl of the Buffer Solution with 25 µl of the Substrate to prepare 50 µl of Working Solution. Unused Working Solution can be aliquoted and stored for up to 24 hours at 4°C in the dark.
- **Standards:** Label 7 tubes with 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.05 mg/ml, and 0.025 mg/ml. Add 100 µl, 80 µl, 60 µl, 40 µl, 20 µl, 10 µl, and 5 µl of Standard (0.5 mg/ml) to the 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.05 mg/ml, and 0.025 mg/ml tubes respectively, followed by 0 µl, 20 µl, 40 µl, 60 µl, 80 µl, 90 µl, and 95 µl of double-distilled water, to prepare Standard Dilutions with concentrations 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.05 mg/ml, and 0.025 mg/ml. These volumes are summarized in the following table:

Standard Dilution (mg/ml)	**0.5**	**0.4**	**0.3**	**0.2**	**0.1**	**0.05**	**0.025**	**0**
0.5 mg/ml Standard (µl)	100	80	60	40	20	10	5	0
Double-distilled water (µl)	0	20	40	60	80	90	95	100

For the blank, or 0 mg/ml standard, use pure double-distilled water. The volume of each standard will be 100 µl.

B. Assay Procedure

1. Assign microplate wells for each standard, sample, and blank. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 5 µl of each standard dilution to the corresponding standard wells.
3. Add 5 µl of sample to the sample wells.
4. Add 5 µl of double-distilled water to the blank wells.
5. Add 50 µl of Working Solution to each well and mix thoroughly with a microplate reader for 30 seconds.
6. Incubate for 15 minutes at 37°C.
7. Add 150 µl of Chromogenic Reagent immediately and mix thoroughly.
8. Measure the OD values of each well at 520 nm with a microplate reader.

C. Calculation of Results

Average the readings for each standard. Subtract the mean OD value of the blank standard (0 mg/ml) from all standard readings. This is the absolute OD value.

Plot the standard curve, using the absolute OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Alkaline phosphatase in each sample well can be derived with the following formulae:

One King Unit is defined as the amount of enzyme required to release 1 mg of phenol from the substrate in 15 minutes. 1 King unit/100mL =7.14 U/L.

1. Serum (Plasma) samples:

$$\text{ALP activity (King unit/100 mL)} = \text{F} \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{blank}} - \text{b}) \times \text{V}_{\text{Sample}}}{\text{a}}$$

2. Tissue samples:

Alkaline phosphatase activity in tissue samples can be calculated according to total protein concentration (which must be assayed separately).

$$\text{ALP activity (King unit/ g Protein)} = \text{F} \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{blank}} - \text{b})}{\text{a} \times \text{C}_{\text{Protein}}}$$

where:

Absolute OD	$\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}$
$\text{OD}_{\text{Sample}}$	OD value of sample
OD_{Blank}	OD value of control
V_{Sample}	Volume of sample per King unit (100 ml)
$\text{C}_{\text{Protein}}$	Concentration of protein in sample (g Protein /ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.